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MINOCYCLINE INHIBITS BRAIN INFLAMMATION AND ATTENUATES SPONTANEOUS RECURRENT SEIZURES FOLLOWING PILOCARPINE-INDUCED STATUS EPILEPTICUS

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Abstract—Mounting evidence suggests that brain inflammation mediated by glial cells may contribute to epileptogenesis. Minocycline is a second-generation tetracycline and has potent antiinflammatory effects independent of its antimicrobial action. The present study aimed to investigate whether minocycline could exert antiepileptogenic effects in a rat lithium-pilocarpine model of temporal lobe epilepsy. The temporal patterns of microglial and astrocytic activation were examined in the hippocampal CA1 and the adjacent cortex following pilocarpine-induced status epilepticus (SE). These findings displayed that SE caused acute and persistent activation of microglia and astrocytes. Based on these findings, Minocycline was administered once daily at 45 mg/kg for 14 days following SE. Six weeks after termination of minocycline treatment, spontaneous recurrent seizures (SRS) were recorded by continuous video monitoring. Minocycline inhibited the SE-induced microglial activation and the increased production of interleukin-1β and tumor necrosis factor-α in the hippocampal CA1 and the adjacent cortex, without affecting astrocytic activation. In addition, Minocycline prevented the SE-induced neuronal loss in the brain regions examined. Moreover, minocycline significantly reduced the frequency, duration, and severity of SRS during the two weeks monitoring period. These results demonstrated that minocycline could mitigate SE-induced brain inflammation and might exert disease-modifying effects in an animal model of temporal lobe epilepsy. These findings offer new insights into deciphering the molecular mechanisms of epileptogenesis and exploring a novel therapeutic strategy for prevention of epilepsy.

Key words: minocycline, epileptogenesis, microglia, astrocytes, cytokine.

INTRODUCTION

Epileptogenesis, i.e., the process leading to epilepsy, is a common sequel of brain insults such as brain injury, cerebrovascular disease, or status epilepticus (SE) (Pitkanen and Sutula, 2002). Such brain insults are typically followed by a latent period, during which the brain undergoes a cascade of morphological and functional alterations over month to years before the onset of chronic epilepsy. Pharmacological intervention of epileptogenic process during this period may prevent or modify epilepsy development (Pitkanen and Lukasiuk, 2011). However, currently available antiepileptic drugs could only alleviate seizure symptoms, and far failed to prevent epileptogenesis (Hitiris and Brodie, 2006). Therefore, there is an urgent need to develop antiepileptogenic drugs by targeting the mechanisms underlying epileptogenic process (Loscher and Brandt, 2010).

Accumulative evidence suggests that inflammation, involving activated glial cells and increased expression of specific inflammatory mediators, may contribute to epileptogenesis (Ravizza et al., 2011; Vezzani et al., 2013b; Xu et al., 2013). Extensive activated glial cells, including microglia and astrocytes, have been well described in patients with drug-resistant epilepsy and in various animal models of seizures (Vezzani et al., 2008, 2013a; Devinsky et al., 2013). In the epileptic brain, activated microglia and astrocytes exhibit morphological changes and could release a large number of cytokines, including interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α) (Vezzani et al., 2008). Experimental studies with perturbed cytokine system in rodents indicate that these proinflammatory cytokines can alter neuronal excitability and decrease seizure threshold, thus favoring the establishment of a chronic neuronal network hyperexcitability which could generate spontaneous recurrent seizures (SRS) (Vezzani et al., 2011, 2013b). Therefore, compounds with antiinflammatory properties or with an ability to inhibit glial activation may represent a promising strategy to improve therapeutic effects on epilepsy.

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Abbreviations: ANOVA, analysis of variance; Con, Control; con-Mino, control-minocycline; con-Nacl, control-Nacl; EEG, electroencephalographic; ELISA, enzyme-linked immunosorbent assay; GABA, γ-aminobutyric acid; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium-binding adaptor molecule 1; IHC, immunohistochemistry; IL-1, interleukin-1; Mino, minocycline; NeuN, neuronal nuclei; NMDA, N-methyl-D-aspartate; PBS, phosphate-buffered saline; TNF-α, tumor necrosis factor-α; SE, status epilepticus; SE-Mino, SE-minocycline; SRS, spontaneous recurrent seizures.

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Minocycline is a second-generation, semi-synthetic tetracycline and has been used for over 30 years for its antibiotic activities against both Gram-positive and Gram-negative bacteria (Yong et al., 2004; Garrido-Mesa et al., 2013). Minocycline is a small, highly lipophilic molecule and has a superior tissue penetration into the brain and cerebrospinal fluid (Saivin and Houin, 1988). Recent evidence reveals that minocycline has potent anti-inflammatory properties which are independent of its antimicrobial action (Kielian et al., 2007; Garrido-Mesa et al., 2013). Its mechanisms of action in the central nervous system include suppression of microglial activation and reduction of proinflammatory cytokine release (Yong et al., 2004; Kim and Suh, 2009). *In vitro* studies, minocycline could inhibit the rapid activation of microglia and the concomitant production of proinflammatory molecules in response to a variety of stimuli, such as glutamate, N-methyl-D-aspartate (NMDA), and kainite (Tikka et al., 2001; Tikka and Koistinaho, 2001). *In vivo* studies, minocycline's beneficial effects in various models of neurological disease were associated with its inhibitory effects on microglial activation (Yrjanheikki et al., 1998; Popovic et al., 2002; Wu et al., 2002; Yoon et al., 2012). In addition, several recent studies demonstrated that minocycline had anticonvulsant effects on partial seizures and electrical- or chemical-kindled seizures (Wang et al., 2012; Ahmadirad et al., 2013; Beheshti Nasr et al., 2013). Moreover, Abraham et al. reported that minocycline could block the long-term epileptogenic effects of early-life seizures in a "two-hit" seizure model (Abraham et al., 2012). However, up to date, minocycline effects on the development of epileptogenesis in a rat model of temporal lobe epilepsy have not been directly explored.

The present study aimed to investigate whether minocycline could exert antiepileptogenic effects in a rat lithium-pilocarpine model of temporal lobe epilepsy. Because glial cells play active roles in the initiation and maintenance of inflammatory process, the time course of microglial and astrocytic activation were first examined in the hippocampal CA1 and the adjacent cortex following SE. Then, minocycline effects on the SE-induced brain inflammation and the development of SRS were assessed. In addition, the effect of minocycline on the SE-induced neuronal loss was also evaluated.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley (SD) rats (200–250 g, aged 8–10 weeks) were supplied by Chongqing Medical University (Chongqing, China). Rats were housed under specific pathogen-free environment on a fixed 12-h light/dark cycle (light on at 7:00 A.M.) at a constant temperature (24–25 °C) with free access to food and water. All experimental protocols were approved by the Ethics Committee of Chongqing Medical University and confirmed to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications NO.80–23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

Induction of SE by lithium-pilocarpine

Rats were injected with lithium chloride (127 mg/kg, i.p.; Sigma–Aldrich, St. Louis, MO, USA). Approximately 18 h later, atropine sulfate (1 mg/kg, i.p.) was administered to limit the peripheral effects. Thirty minutes later, rats were administered a single dose of pilocarpine (40 mg/kg, i.p.; Sigma). Behavioral changes were recorded and graded according to Racine's scale (Racine, 1972). The beginning of SE was defined as the onset of ongoing limbic or generalized convulsive seizures without regaining normal behavior between seizures (Jung et al., 2006; Chu et al., 2008). Rats developing a self-sustained SE with generalized convulsive seizures were included in subsequent experiments. Diazepam (10 mg/kg, i.p.) was administered after 90 min of SE to terminate generalized convulsive seizures. Diazepam application was repeated after 15 min and 6 h to prevent recurrence of SE and to reduce mortality. Control rats were treated with lithium, methyl-scopolamine, and diazepam as experimental rats but 0.9% Nacl instead of pilocarpine. Following SE, all rats were injected with 0.9% Nacl (5 ml, twice a day, i.p.) and fed with eggs over a couple of days until they commenced to eat their normal pellets.

Minocycline administration

Minocycline hydrochloride (Sigma) was freshly dissolved in 0.9% Nacl and administered intraperitoneally once daily at a dosage of 45 mg/kg rat body weight, starting immediately after termination of SE. The selection of dose was based on previous studies showing beneficial effects of this dosage in animal models of cerebral brain ischemia, multiple sclerosis, and Parkinson disease (Yrjanheikki et al., 1998, 1999; Popovic et al., 2002; Wu et al., 2002).

Experimental protocol

Two experiments were conducted in this study. In experiment 1, the activated profiles of microglia and astrocytes were detected in the hippocampal CA1 and the adjacent cortex following SE by immunohistochemistry (IHC). Rats were randomly allocated into four groups: the control (Con, rats without SE), the SE 3 days (SE-3d), the SE 7 days (SE-7d), and the SE 14 days (SE-14d) group. Each group had five rats.

In experiment 2, minocycline effects on the SE-induced brain inflammation and the development of SRS were assessed. In addition, electroencephalographic (EEG) recording was performed to assess minocycline effect on electrographic seizures during the first several days after pilocarpine injection. Rats with SE were randomly allocated into two groups: (1) the SE-minocycline (SE-Mino) group, which received minocycline immediately after termination of SE, followed by once daily minocycline treatment (45 mg/kg, i.p.) for the subsequent 13 days; (2) the SE-Nacl group, which received 0.9% Nacl instead of minocycline once daily for 14 consecutive days. The control rats without SE were also randomly allocated into two groups: the control-Nacl (con-Nacl) group and the